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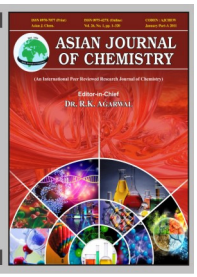

Simultaneous Assay of Three Diterpenoids with One Marker in Semen *Euphorbiae pulveratum*

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Euphorbia is a widely used traditional Chinese medicine (TCM) from the dry mature seed of *Euphorbia lathyris* L. which can enhance diuresis. Diterpenoids such as euphorbia factor L_1, euphorbia factor L_2 and euphorbia factor L_3, are the main active ingredients. In present work, the researchers attempted to establish a quantitative method for the simultaneous assay of three diterpenoids with one marker in semen *Euphorbiae pulveratum* and then validated its feasibility and durability in controlling the quality of semen *Euphorbiae pulveratum*. High-performance liquid chromatography method was used for the experiment. The retention value relative to the chromatographic peak of the component to be measured was determined and then the relative correction factor and the relative retention value were computed. Finally, the relative error was established to be less than 5 % by comparing the quantitative results of quantitative analysis of multi-components by single marker (QAMS) with those of the external standard method. Overall, the usage of QAMS is feasible for the simultaneous determination of euphorbia factor L_1, euphorbia factor L_2 and euphorbia factor L_3, as no significant differences were observed between the measured values and the calculated values.

Keywords: Semen *Euphorbiae pulveratum*, Quantitative analysis of multi-components by single marker.

INTRODUCTION

Euphorbia comes from the dry mature seed of *Euphorbia lathyris* L. and belongs to the family of euphorbiaceae. It can expel water retention with drastic purgative effects, namely, breaking up the static blood and eliminating masses. Euphorbia can be used to treat tinea, warts, anuria, constipation, edema, phlegm, stagnation, fullness and amenorrhea caused by blood stasis[1]. In recent years, reports of its use for the treatment of leukemia, esophageal cancer and skin cancer have been published[2]. The main chemical compositions of euphorbia are fatty oil, coumarin and diterpenes[3]. Among these components, diterpenes mainly consist of euphorbia factor L_1, euphorbia factor L_2 and euphorbia factor L_3. Simultaneous determination of several components to control the quality of drugs and herbs are becoming more and more popular[4,5], several components can reflect the quality of measured object better than one component quantitative assay of these three diterpenes in *Euphorbia lathyris* has been reported[6-8]. But these methods are tedious and complex, researchers have been looking for a simple method to determinate the representative component can simultaneously control multiple components in traditional Chinese medicine.

Quantitative analysis of multi-components by single marker is a method based on the principle that the content of composition is proportional to the detector response within a certain linear range. In quantitative analysis of multi-components by single marker (QAMS), a typical effective ingredient designated as an internal reference substance is used to calculate the content of other components using the relative correction factor established between the component to be measured and the internal reference substance[9]. This method has been widely applied in quality analysis of flavonoids[10], saponins[11], phenolic acids[12] and different types of compounds in traditional Chinese medicine[13,14]. The establishment of QAMS for the three diterpenes simultaneously controls the content of multiple components in semen *Euphorbiae pulveratum* and provides scientific basis for research on quality standard of semen *Euphorbiae pulveratum*.

EXPERIMENTAL

The following were the instruments and reagents used in the experiment: LC-2010AHT high-performance liquid chromatograph (Shimadzu Corporation), PDA detector (Shimadzu Corporation), Waters 1525-2489 high-performance liquid chromatograph (Waters Corporation), LC-20AT high-perfor-

mance liquid chromatograph (Shimadzu Corporation), Mettler Toledo-XS205 1/100000 balance (Swiss Mettler Toledo Company), BS 210S 1/10000 electronic balance (Beijing Sartorius), KQ-250DV NC ultrasonic cleaner (Kunshan Ultra-sonic Instruments Co., Ltd.), Agilent Zorbax-SIL high-performance liquid chromatography (HPLC) column (4.6 mm × 250 mm, 5 μm), Hypersil NH_2 HPLC column (4.6 mm × 250 mm, 5 μm) and Agilent Zorbax NH_2 HPLC column (4.6 mm × 250 mm, 5 μm).

The substances used in the experiment were as follows: reference substance of euphorbia factor L_1 (National Institutes for Food and Drug Control (NIFDC), China, Batch No. 111789-200901, purity: 99.3 %), reference substance of euphorbia factor L_2 (National Institutes for Food and Drug Control (NIFDC), China, Batch No. 111790-200901, purity: 98.5 %), reference substance of euphorbia factor L_3 (National Institutes for Food and Drug Control (NIFDC), China, Batch No. 111791-200901, purity: 98.6 %), *n*-hexane (Tianjin Kemiou Chemical Reagent Co., Ltd., chromatographically pure), ethyl acetate (Xilong Chemical Joint Stock Company, Chromatographically pure) and acetonitrile (Dikma Technologies Inc., chromatogra-phically pure).

In the experiment, 27 batches of semen *Euphorbiae pulveratum* samples were used: 3 of them were prepared using the traditional method, 3 of them were prepared using pharmacopoeia method, 3 of them were prepared using dilution method, 3 of them were prepared using fat oil extraction and oiling, 3 of them were prepared using a manual screw machine and the remaining 12 batches were prepared using a hot-pressing machine.

Chromatographic requirements: The chromatographic requirements were as follows: SHIMADZU LC-2010AHT high-performance liquid chromatograph, PDA detector, Agilent Zorbax-SIL HPLC column (4.6 mm × 250 mm, 5 μm), *n*-hexane-ethyl acetate-acetonitrile = 83.5:14:2.5, 30 °C column temperature, 275 nm detection wavelength, 1 mL min^{-1} volume flow rate, 3 μL injection volume and 20 min detection time. The chromatograms are presented in Fig. 1.

RESULTS AND DISCUSSION

Exactly 3.46 mg of the reference substance of euphorbia factor L_1, 2.17 mg of the reference substance of euphorbia factor L_2 and 4.95 mg of the reference substance of euphorbia factor L_3 were placed into a 100 mL volumetric flask and then ethyl acetate was added until the set volume was reached. After shaking the flask, the reference substance of the mixture of euphorbia factor L_1, euphorbia factor L_2 and euphorbia factor L_3 was obtained. The mass concentrations were 0.0346, 0.0217 and 0.0495 mg mL^{-1}, respectively.

Preparation of the sample solution: About 0.2 g of semen *Euphorbiae pulveratum* from each batch was placed into a conical flask with plug and then 25 mL ethyl acetate was added. The flask was sealed and weighed. After ultrasonic treatment (power 250 W, frequency 25 kHz) for 20 min, the flask was cooled and then weighed. Ethyl acetate was added to supplement the lost weight, after which the flask was shaken and then filtered. The filtrate is the sample solution.

Investigation of linear relationship: A total of 1, 2, 3, 4 and 5 μL of the solution of the reference substance of the mixture of euphorbia factor were injected. Determination was performed under the above chromatographic conditions. With the integral value of peak area as a vertical coordinate (Y) and the amount of compound (μg) as abscissa (X), the standard curve was drawn. Finally, linear regression was performed and then the standard curve equation and linear range were obtained. The results are shown in Table-1.

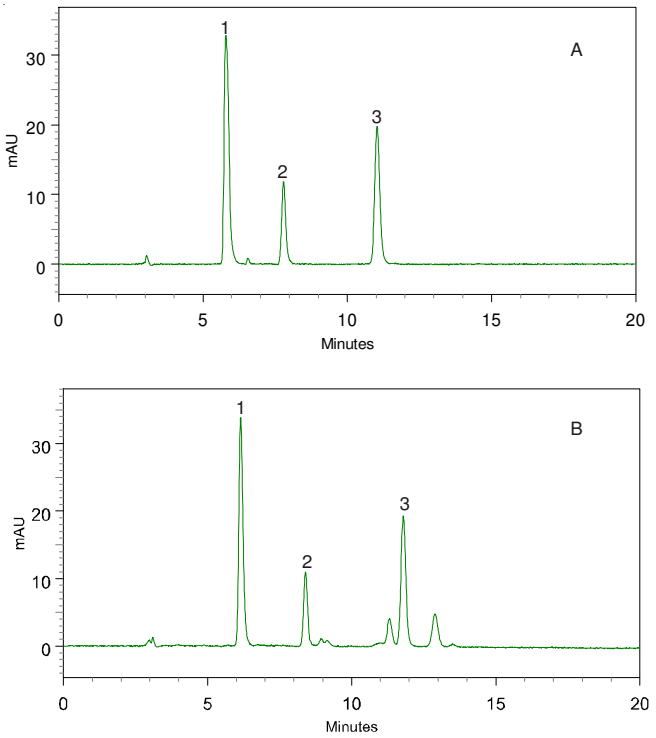

Fig. 1. Chromatograms of the three diterpenoids in semen *Euphorbiae pulveratum* (a) The reference substance of the mixture of euphorbia factor L_1, euphorbia factor L_2 and euphorbia factor L_3 (b) Semen *Euphorbiae pulveratum* sample 1) euphorbia factor L_3, 2) euphorbia factor L_2, 3) euphorbia factor L_1

TABLE-1
REGRESSION EQUATION AND LINEAR RANGE OF THE THREE DITERPENES

Compound	Regression equation	Correlation coefficient (r)	Linear range (μg)
Euphorbia factor L_1	Y=1405072.25X+3795.3	0.9999	0.0346-0.1730
Euphorbia factor L_2	Y=1082460.83X+2860.0	0.9999	0.0217-0.1085
Euphorbia factor L_3	Y=1536646.46X+6853.4	1.0000	0.0495-0.2475

Precision test: Exactly 5 μL of the solution of the reference substance of the mixture of euphorbia factor was injected six times under the above chromatographic conditions. The peak area values of euphorbia factor L_1, euphorbia factor L_2 and euphorbia factor L_3 were determined. The RSD values were 1.18, 2.75 and 1.16 %. Results indicate that the instrument had good precision.

Reproducibility test: About six portions of semen *Euphorbiae pulveratum* from the same batch were weighed to prepare the sample solution according to the corresponding method. The test was performed under the above chromatographic conditions, with an injection volume of 3 μL. The

integral values of the peak area of euphorbia factor L_1, euphorbia factor L_2 and euphorbia factor L_3 were measured. The RSD values were 0.95, 1.87 and 1.05 %. The results show that the reproducibility of the test was good.

Stability test: Exactly one portion of semen *Euphorbiae pulveratum* was weighed to prepare the sample solution according to the corresponding method. Sampling was performed at 0, 2, 4, 8 and 12 h and the sampling volume for each was 3 μL. The integral values of the peak areas of euphorbia factor L_1, euphorbia factor L_2 and euphorbia factor L_3 were measured. RSD values were 2.57, 2.99 and 2.56 %. The results show that the sample solution was stable within 12 h.

Recovery test: Up to six portions of semen *Euphorbiae pulveratum* with known content from the same batch, each about 0.1 g, was added to the 10 mL mixed standard solution. The sample solution was prepared according to the corresponding method. Sampling was performed under the above chromatographic conditions and the injection volume was 3 μL. Their average recoveries were 100.2 % (RSD = 2.35 %), 99.5 % (RSD = 2.32 %) and 104.0 % (RSD = 1.82 %), respectively. The results show that the method had good accuracy.

Determination of relative correction factor for the three diterpenes: A total of 1, 2, 3, 4 and 5 μL reference solution of the mixed euphorbia factor were injected and the peak area of each component was determined. With euphorbia factor L_1 as internal reference substance, the relative correction factor of euphorbia factor L_2 and euphorbia factor L_3 to the internal reference substance were calculated according to the relative correction factor formula (Table-2).

TABLE 2
RELATIVE CORRECTION FACTOR OF THE THREE DITERPENES

Sampling Volume (μ L)	Relative correction factor	
	$f_{\text{euphorbia factor L2/euphorbia factor L1}}$	$f_{\text{euphorbia factor L3/euphorbia factor L1}}$
1.0	1.260	0.9218
2.0	1.238	0.8909
3.0	1.277	0.9006
4.0	1.247	0.8902
5.0	1.267	0.8762
Mean	1.26	0.90
RSD (%)	1.13	1.68

Note : $f_{i/s} = \frac{f_i}{f_s} = \frac{A_i / C_i}{A_s / C_s} = \frac{A_i \times C_s}{A_s \times C_i}$

*where C_s is the concentration of the reference substance of the internal reference substance S, A_s is the peak area of the reference substance of the internal reference substance S, A_i is the peak area of component *i* which will be tested, and C_i is the concentration of the reference substance of component *i*

Durability of relative correction factor

Effect of different instruments upon the relative correction factor: Agilent Zorbax-SIL HPLC column (4.6 mm × 250 mm, 5 μm) was used to study the effect of three high-performance liquid chromatographs on the different relative correction factors. The results showed that the relative correction factor of euphorbia factor L_2 and euphorbia factor L_3 to the internal standard had good reproducibility (RSD < 5 %) (Table-3).

TABLE-3
EFFECT OF DIFFERENT INSTRUMENTS ON THE RELATIVE CORRECTION FACTOR (n = 2)

Instrument	Relative correction factor	
	$f_{\text{euphorbia factor L2/euphorbia factor L1}}$	$f_{\text{euphorbia factor L3/euphorbia factor L1}}$
Shimadzu LC-2010ATH	1.260	0.8991
Waters 1525-2489	1.317	0.9019
Shimadzu LC-20AT	1.355	0.9103
Mean	1.31	0.90
RSD (%)	3.64	0.64

Effect of different columns on the relative correction factor: The SHIMADZU LC-2010ATH HPLC system was used to study the effects of the three different chromatographic columns (4.6 mm × 250 mm, 5 μm) on the relative correction factor. These columns were Agilent Zorbax-SIL HPLC column, elite hypersil NH_2 HPLC column and Agilent Zorbax NH_2 HPLC column. The results showed that the relative correction factor of euphorbia factor L_2 and euphorbia factor L_3 to the internal standard had good reproducibility (RSD < 5 %) (Table-4).

TABLE-4
EFFECT OF DIFFERENT COLUMNS ON THE RELATIVE CORRECTION FACTOR (n = 2)

Column	Relative correction factor	
	$f_{\text{euphorbia factor L2/euphorbia factor L1}}$	$f_{\text{euphorbia factor L3/euphorbia factor L1}}$
Agilent Zorbax-SIL	1.269	0.8913
Elite Hypersil NH_2	1.257	0.9259
Agilent Zorbax NH_2	1.319	0.9285
Mean	1.28	0.91
RSD (%)	2.56	2.27

Effect of different flow rates on the relative correction factor: The SHIMADZU LC-2010ATH system and the Agilent Zorbax-SIL HPLC column were used to study the effect of different flow rates on the relative correction factor. The results showed that the relative correction factor of euphorbia factor L_2 and euphorbia factor L_3 to the internal standard had good reproducibility (RSD < 5 %) (Table-5).

TABLE 5
EFFECT OF DIFFERENT FLOW RATES ON THE RELATIVE CORRECTION FACTOR (n = 2)

Flow rate (mL min^{-1})	Relative correction factor	
	$f_{\text{euphorbia factor L2/ euphorbia factor L1}}$	$f_{\text{euphorbia factor L3/ euphorbia factor L1}}$
0.9	1.184	0.8898
1.0	1.206	0.9141
1.1	1.258	0.9114
Mean	1.22	0.91
RSD (%)	3.12	1.47

Effect of different column temperatures on the relative correction factor: The SHIMADZU LC-2010ATH system and the Agilent Zorbax-SIL HPLC column were used to study

the effect of different column temperatures on the relative correction factor. The results showed that the relative correction factor of euphorbia factor L_2 and euphorbia factor L_3 to the internal standard had good reproducibility (RSD < 5 %) (Table-6).

TABLE-6
EFFECT OF DIFFERENT COLUMN TEMPERATURES ON THE RELATIVE CORRECTION FACTOR (n = 2)

Column Temperature (°C)	Relative correction factor	
	$f_{\text{euphorbia factor L2/euphorbia factor L1}}$	$f_{\text{euphorbia factor L3/euphorbia factor L1}}$
25	1.208	0.9486
30	1.195	0.9112
35	1.252	0.9109
Mean	1.22	0.92
RSD (%)	2.42	2.35

Effect of different detection wavelengths on the relative correction factor: The SHIMADZU LC-2010ATH system and the Agilent Zorbax-SIL HPLC column were used to study the effect of different detection wavelengths on the relative correction factor. The results showed that the relative correction factor of euphorbia factor L_2 and euphorbia factor L_3 to the internal standard had good reproducibility (RSD < 5 %) (Table-7).

TABLE 7
EFFECT OF DIFFERENT DETECTION WAVELENGTHS ON THE RELATIVE CORRECTION FACTOR (n = 2)

Wavelength (nm)	Relative correction factor	
	$f_{\text{euphorbia factor L2/ euphorbia factor L1}}$	$f_{\text{euphorbia factor L3/ euphorbia factor L1}}$
270	1.282	0.9442
275	1.189	0.8803
280	1.285	0.8789
Mean	1.25	0.90
RSD (%)	4.38	4.14

Effect of different mobile phases on the relative correction factor: The SHIMADZU LC-2010ATH system and the Agilent Zorbax-SIL HPLC column were used to study the effect of different mobile phases on the relative correction factor. The results showed that the relative correction factor of euphorbia factor L_2 and euphorbia factor L_3 to the internal standard had good reproducibility (RSD < 5 %) (Table-8).

Position of the chromatographic peak of the component to be tested

Investigation of the relative retention value (RT_R): RT_R was used to position the chromatographic peak. The reproducibility of RT_R in different instruments and different chromatographic columns was investigated (Tables 9 and 10).

TABLE-9
EFFECT OF DIFFERENT INSTRUMENTS ON RT_R

Instrument	Relative retention value	
	$RT_{R\ \text{euphorbia factor L2/euphorbia factor L1}}$	$RT_{R\ \text{euphorbia factor L3/ euphorbia factor L1}}$
Shimadzu LC-2010ATH	0.6844	0.4529
Waters 1525-2489	0.6642	0.4772
Shimadzu LC-20AT	0.7255	0.4880
Mean	0.69	0.47
RSD (%)	4.52	3.80

TABLE-10
EFFECT OF DIFFERENT CHROMATOGRAPHIC COLUMNS ON RT_R

Column	Relative retention value	
	$RT_{R\ \text{euphorbia factor L2/ euphorbia factor L1}}$	$RT_{R\ \text{euphorbia factor L3/ euphorbia factor L1}}$
Agilent Zorbax-SIL	0.7307	0.5413
Elite Hypersil NH_2	0.9003	0.6550
Agilent Zorbax NH_2	0.7307	0.5413
Mean	0.79	0.58
RSD (%)	12.44	11.34

Calculation of RT_R: The SHIMADZU LC-2010ATH system and the Agilent Zorbax-SIL HPLC column were used. The relative retention value of euphorbia factor L_2 and euphorbia factor L_3 to the internal standard were determined (Table-11).

TABLE-11
RELATIVE RETENTION VALUE

No.	Relative retention value	
	$RT_{R\ \text{euphorbia factor L2/ euphorbia factor L1}}$	$RT_{R\ \text{euphorbia factor L3/ euphorbia factor L1}}$
1	0.6633	0.4767
2	0.6637	0.4766
3	0.6633	0.4763
4	0.6635	0.4760
5	0.6654	0.4778
6	0.6674	0.4788
7	0.6690	0.4797
8	0.6682	0.4786
9	0.6681	0.4785
10	0.6671	0.4781
Mean	0.67	0.48
RSD (%)	0.34	0.26

Comparison of results between QAMS and external standard method: Twenty-seven batches of Semen *Euphorbiae pulveratum* samples were prepared and HPLC method was used. Measured values obtained from external standard method were compared with the calculated values from QAMS to evaluate the scientific accuracy of QAMS. The results show

Table-8
EFFECT OF DIFFERENT MOBILE PHASES ON THE RELATIVE CORRECTION FACTOR (n = 2)

Mobile Phase				Relative correction factor	
A	B	C	Ratio	$f_{\text{euphorbia factor L2/ euphorbia factor L1}}$	$f_{\text{euphorbia factor L3/ euphorbia factor L1}}$
Hexane	Ethyl acetate	Acetonitrile	84:14:2	1.211	0.8947
Hexane	Ethyl acetate	Acetonitrile	83.5:14:2.5	1.271	0.8778
Hexane	Ethyl acetate	Acetonitrile	83:14:3	1.250	0.8916
Mean		1.24	0.89		
RSD (%)		2.44	1.01		

that values from the two methods with three components and peak location did not exhibit significant difference and the relative error is less than 5 %, suggesting that QAMS has good reliability (Tables 12 and 13).

The high oil content of the plants can be used as raw material for biodiesel, euphorbia also has this feature[15]. Euphorbia contains fatty oil as its main toxic component, which can produce a series of gastrointestinal side effects. Modern processing, such as cold pressing or hot pressing, can be used to remove part of the fatty oil. The percentage of fatty oil in euphorbia is up to 43.66 to 45.7 %[16]. The chinese pharmacopoeia 2010 edition requires that the euphorbia factor should contain 18 to 20 % of fatty oil[1], in which the content of fatty oil decreases by more than half. The pharmacological action of semen *Euphorbiae pulveratum* is different from that of euphorbia. An inflammatory ascite model was constructed by intraperitoneal injection of 0.6 % acetic acid and intragastric administration of a suspension of semen *Euphorbiae pulveratum* to mice. Results show that semen *Euphorbiae pulveratum* can significantly reduce the weight of ascite mice, reduce edema and enhance diuresis[17]. Acute toxicological studies on euphorbia, fatty oil of euphorbia and semen *Euphorbiae pulveratum* show that liquid stools were excreted by mice in the semen *Euphorbiae pulveratum* group when they were administered with 39.96 g kg^{-1} of the drugs, which is equivalent to 2400 times of the clinical dose. However, symptoms are milder than those in the euphorbia factor group and fatty oil group, which showed that the toxicity of semen *Euphorbiae pulveratum* decreased significantly. Within a certain range, no significant difference of toxicity was observed between some dose ranges of semen *Euphorbiae pulveratum* with different contents of oil; the important organs of mice did not show signs of obvious pathological damage; and the organ index between the two groups exhibited no significant effects or death, indicating that the safety range of semen *Euphorbiae pulveratum* was larger[18]. The above findings show that reduction of oil and preparation of euphorbia as cream in clinical applications and in controlling the content of fatty oil in semen *Euphorbiae pulveratum* have a sound scientific basis.

The study shows that euphorbia can enhance diuresis, which is mainly caused by diterpenoids[19]. Euphorbia factor L_1, euphorbia factor L_2 and euphorbia factor L_3 accounted for a larger proportion of diterpenoids[7]. Under the chromatographic conditions set up in this study, the three components accounted for about 90 %, which can be used for accurate quality control of semen *Euphorbiae pulveratum*.

TABLE-12
RETENTION TIME OF THE COMPONENT TO BE TESTED MEASURED USING QAMS AND EXTERNAL STANDARD METHOD (min)

No.	Batch No.	Euphorbia factor L_1	Euphorbia factor L_2			Euphorbia factor L_3		
		External standard method	External standard method	QAMS	Relative error (%)	External standard method	QAMS	Relative error (%)
1	20120315a	12.08	7.872	8.096	2.84	5.635	5.800	2.93
2	20120316b	12.07	7.851	8.088	3.02	5.845	5.795	-0.86
3	20120317c	12.12	8.053	8.117	0.80	5.941	5.815	-2.12
4	20120320a	12.21	8.004	8.181	2.22	5.877	5.861	-0.27
5	20120321b	12.03	7.840	8.059	2.80	5.824	5.774	-0.86
6	20120322c	12.04	7.849	8.067	2.77	5.803	5.779	-0.41
7	20120404a	12.10	8.021	8.110	1.11	5.931	5.810	-2.04
8	20120404b	12.14	8.053	8.131	0.97	5.952	5.825	-2.13
9	20120404c	12.12	8.053	8.117	0.80	5.941	5.815	-2.12
10	20120406a	12.13	8.032	8.124	1.14	5.920	5.820	-1.69
11	20120406b	12.15	8.043	8.138	1.19	5.931	5.831	-1.69
12	20120406c	12.12	8.032	8.117	1.06	5.931	5.815	-1.95
13	20120512a	12.15	8.032	8.138	1.33	5.925	5.830	-1.59
14	20120513b	12.24	8.043	8.203	1.99	5.941	5.877	-1.08
15	20120514c	12.09	8.021	8.102	1.01	5.941	5.805	-2.30
16	20120805a	12.14	8.021	8.131	1.37	5.931	5.825	-1.78
17	20120806a	12.20	8.085	8.174	1.10	5.973	5.856	-1.96
18	20120807a	12.31	7.968	8.246	3.49	5.909	5.907	-0.03
19	20120808a	12.93	8.437	8.660	2.64	6.197	6.204	0.11
20	20120809a	12.61	8.171	8.445	3.36	6.016	6.050	0.57
21	20120910a	12.64	8.224	8.467	2.95	6.037	6.066	0.48
22	20120912a	12.58	8.203	8.431	2.78	6.037	6.040	0.05
23	20120918a	12.62	8.203	8.453	3.04	6.027	6.056	0.48
24	20120918b	12.47	8.149	8.353	2.50	6.005	5.984	-0.35
25	20120918c	12.44	8.160	8.331	2.10	6.016	5.969	-0.78
26	20120922a	12.35	8.032	8.274	3.01	5.931	5.928	-0.06
27	20121010a	12.56	8.160	8.417	3.15	6.005	6.030	0.42

*Note: relative error = (QAMS – external standard method)/external standard method

TABLE-13
CONTENT OF THE COMPONENT TO BE TESTED MEASURED USING QAMS AND EXTERNAL STANDARD METHOD (mg g^{-1})

No.	Batch No.	Euphorbia Factor L_1	Euphorbia factor L_2			Euphorbia factor L_3		
		External standard Method	External Standard Method	QAMS	Relative Error (%)	External Standard Method	QAMS	Relative Error (%)
1	20120315a	0.2666	0.1677	0.1665	-0.75	0.3419	0.3404	-0.43
2	20120316b	0.2716	0.1878	0.1853	-1.33	0.3593	0.3571	-0.60
3	20120317c	0.2549	0.1472	0.1471	-0.09	0.3093	0.3091	-0.07
4	20120320a	0.2295	0.1413	0.1409	-0.26	0.2947	0.2939	-0.26
5	20120321b	0.2298	0.1509	0.1498	-0.71	0.3080	0.3064	-0.51
6	20120322c	0.2262	0.1462	0.1454	-0.57	0.2923	0.2915	-0.28
7	20120404a	0.2482	0.1557	0.1548	-0.58	0.3517	0.3486	-0.87
8	20120404b	0.2471	0.1489	0.1484	-0.30	0.3395	0.3371	-0.71
9	20120404c	0.3152	0.1945	0.1926	-0.98	0.4084	0.4058	-0.64
10	20120406a	0.2858	0.1864	0.1843	-1.09	0.3838	0.3810	-0.72
11	20120406b	0.3083	0.2040	0.2013	-1.30	0.4184	0.4150	-0.82
12	20120406c	0.2951	0.1946	0.1922	-1.21	0.4150	0.4111	-0.94
13	20120512a	0.2110	0.1264	0.1266	0.18	0.2938	0.2919	-0.65
14	20120513b	0.2135	0.1325	0.1324	-0.14	0.2959	0.2941	-0.63
15	20120514c	0.2783	0.1701	0.1690	-0.67	0.3479	0.3467	-0.35
16	20120805a	0.2638	0.1635	0.1624	-0.63	0.3438	0.3421	-0.50
17	20120806a	0.2637	0.1666	0.1654	-0.75	0.3385	0.3371	-0.42
18	20120807a	0.2388	0.1466	0.1461	-0.34	0.3222	0.3204	-0.58
19	20120808a	0.5230	0.2655	0.2628	-1.04	0.5778	0.5747	-0.55
20	20120809a	0.5353	0.2535	0.2515	-0.81	0.5242	0.5233	-0.19
21	20120910a	0.4621	0.2115	0.2108	-0.31	0.4812	0.4803	-0.20
22	20120912a	0.5100	0.2305	0.2294	-0.49	0.5006	0.4999	-0.12
23	20120918a	0.4344	0.2255	0.2237	-0.77	0.4940	0.4917	-0.45
24	20120918b	0.5231	0.1870	0.1881	0.61	0.4422	0.4439	0.40
25	20120918c	0.5716	0.1966	0.1976	0.52	0.4539	0.4560	0.48
26	20120922a	0.5213	0.3059	0.3011	-1.56	0.7076	0.6997	-1.12
27	20121010a	0.4785	0.2832	0.2790	-1.48	0.6251	0.6190	-0.98

When determining the chromatographic conditions, two mobile phases, namely, methanol-water and *n*-hexane-ethyl acetate-acetonitrile, were selected and measured. The two phases were able to achieve good baseline separation. However, when methanol-water was used as the mobile phase, the time spent for the peak of the three diterpenoids was 85 min. A longer time is required for the methanol-water phase and methanol is toxic, so *n*-hexane-ethyl acetate-acetonitrile was chosen as the mobile phase. Through repeated tests, the rate of the mobile phase was determined as 83.5:14:2.5 (*n*-hexane: ethyl acetate:acetonitrile). Under these conditions, chromatographic peak and separation effect are good, detection time is short and ethyl acetate is less toxic.

Given the less polarity of diterpenoids, the separation effect is not ideal when reversed-phase HPLC is used. In the literature, normal-phase HPLC system is used. In this paper, amino column and silica gel column were compared. The former has a short service life and unstable separation among other attributes, so silica gel column chromatography was chosen. Experiments show that the separation effect of using normal phase column is better than that of reversed phase column, which is in agreement with published data[7].

Conclusion

In this experiment, HPLC was used to establish a quantitative method for simultaneous assay of euphorbia factor L_1, euphorbia factor L_2 and euphorbia factor L_3. Furthermore, when using QAMS, euphorbia factor L_1 was selected as internal reference substance. The relative correction factors of euphorbia factor L_2 and euphorbia factor L_3 to the internal reference substance were established to determine a method using the relative retention value method to locate the chromatographic peak of the component to be measured. The relative error was less than 5 % and this value was achieved by comparing the quantitative results of QAMS with those of the external standard method. No significant difference was observed between the two methods.

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ERRATUM

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NOTE

Pyridinium Chlorochromate Catalyzed Oxidation of Toluenes to Aromatic Carboxylic Acids with Molecular Oxygen in Sub-critical Water

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Please note the following corrected Table-1 as follows:

TABLE-1
OXIDATION OF SUBSTITUTED TOLUONES TO CARBOXYLIC ACID IN THE PRESENCE IN THE PRESENCE OF MOLECULAR OXYGEN WITH PCC IN SUB-CRITICAL WATER

Entry	Substrate	Product[d]	m.p. (°C) [ref.]	Yield (%) [P_{O_2} (bar)][a,b]				
				A[c]	5	10	15	20
1	C_6H_5Me	C_6H_5COOH	120-121 (121-123)[16]	72	74	78	82	88
2	*p*-$CH_3C_6H_4Me$	*p*-$COOHC_6H_4COOH$	(210-211)[16] (decomp.)	73	78	80	87	90
3	*m*-BrC_6H_4Me	*m*-BrC_6H_4COOH	154-156 (155-158)[16]	72	83	90	95	95
4	*m*-FC_6H_4Me	*m*-FC_6H_4COOH	(122-124)[16]	85	90	93	95	95
5	*m*-CNC_6H_4Me	*m*-CNC_6H_4COOH	(220-222)[16]	90	94	95	95	95
6	*p*-CNC_6H_5CCMe	*p*-CNC_6H_4CCOOH	(217-218)[16]	90	92	94	95	95
7	*p*-BrC_6H_4Me	*p*-BrC_6H_4COOH	(252-254)[16]	75	78	85	90	95
8	*p*-FC_6H_4CMe	*p*-FC_6H_4CCOOH	(186)[16]	83	85	90	93	95
9	*p*-ClC_6H_4Me	*p*-ClC_6H_4COOH	237-239 (238-241)[16]	80	85	88	90	90
10	*p*-$CH_3OC_6H_4Me$	*p*-$CH_3OC_6H_4COOH$	(183-184)[16]	75	82	84	85	85
11	*o*-$CH_3OC_6H_4Me$	*o*-$CH_3OC_6H_4COOH$	(98-100)[16]	72	75	83	85	85

[a]Double equivalents of molecular oxygen, 5 bar, 4.63×10^{-3} mol dm^{-3}; 10 bar, 9.26×10^{-3} mol dm^{-3}; 15 bar, 1.38×10^{-2} mol dm^{-3}; 20 bar, 1.85×10^{-2} mol dm^{-3}
[b]Relative yield based on quantitative analysis
[c]A: Dissolved oxygen in water at atmospheric pressure 1.26×10^{-3} mol dm^{-3}
[d]All products were characterized by IR and ^1H NMR and their physical data compared with literature data[16]. Reactions conditions: Total pressure 60 bar, temperature 120 °C.